Mechanisms of Trap Movement II: Does *Aldrovanda* Close by a Turgor Mechanism? A Question of How Much, Where, and When

By S.E.Williams
Department of Biology
Lebanon Valley College
Annville, PA 17003

Of the three genera of the family *Droseraceae* with movements involved in their trapping mechanisms *Drosera* tentacles and *Dionaea* traps are reported to close by a mechanism involving a relatively rapid increase in wall plasticity on the abaxial (back, outer) side of the tentacles or lobes (Williams, 1991). Strangely *Aldrovanda* trap lobes which are very similar to *Dionaea* lobes are reported to move by a rapid loss of turgor in the adaxial (inner) side of the trap lobes (Ashida 1935, Iijima and Sibaoka, 1981, 1982, 1983, 1984). In their recent book The Carnivorous Plants (p.104) Juniper, Robins and Joel state "Studies on *Dionaea* and *Aldrovanda* have led to two contradictory views being put forward as to how rapid movements occur in these species." This is not quite true since the two mechanisms are not mutually exclusive and it is possible that both a turgor loss on the adaxial (inner) side and acid growth on the abaxial side could occur together and cause the movement in either plant. The evidence for acid growth in *Dionaea* was reviewed last time (Williams, 1991). There is evidence against turgor loss as a mechanism in *Dionaea.* The abaxial (outer) side expands during opening and loses turgor while the adaxial (inner) side shows no significant change in size and remains turgid[1]. The evidence in *Aldrovanda is* more equivocal and requires careful quantitative scrutiny.

Iijima and Sibaoka (1983) give the following evidence:

1. Experiments using rubidium ion as a marker for potassium ion indicate a loss of 0.25% of the trap potassium during an action potential. Loss of potassium and chloride ion is the cause of the turgor loss in the *Mimosa* pulvinus, guard cells and other well studied turgor mechanisms of plant movement.

2. Experiments using rubidium ion indicate potassium is taken up by the traps at a greater rate after trap closure in reopening traps than it is in unclosed traps. This would also be expected in a turgor controlled trap.

3. Traps placed in 200 mM mannitol-APW[2] will produce an action potential when stimulated but will not close. When placed back in a very dilute ionic solution (APW) these traps still remained open. Iijima and Sibaoka (1983) state that this shows that "no ion leakage from the active motor cells was caused by the action potential in 200-mM mannitol-APW which is nearly isotonic to the inner and outer epidermal cells in the motor zones." They also state that "This fact strongly suggests that the solute leakage from the active motor cells necessitates the pressure inside the cells and is induced by bulk flow, not by diffusional flow, between the vacuole and the outside of the cell wall."

Atomic absorption spectrum measurements of K^+ indicate that there is a concentration of 49.5±4.7 mM (about 50 mmole/liter K^+) in the traps (Iijima and Sibaoka (1985). Measurements of trap cell volume indicate that it is about one μliter (= 1.0 mm^3) (Iijima and Sibaoka, 1983) a value that seems reasonable when compared to the dimensions of the trap. From these values it can be computed that there is about 50 nmole of K^+ in a trap.

50 mmole/liter X 10^{-6} liter/μliter = 50 X 10^{-6} mmole/μliter
50 X 10^{-6} mmole/μliter X 10^{9} pmole/mmole = 50 X 10^{3} pmole/μliter or 50 nmole/1 μliter trap

Iijima and Sibaoka (1983) report a loss of 374 pmole of K^+ from a trap based on their Rb^+ measurements. This is about 0.75% of the trap potassium as measured by the atomic absorption spectrophotometer. It is more than the 0.25% estimated from rubidium measurements alone (Iijima and Sibaoka, 1983).

Since the active area of the trap is about 0.38 cm^2 (Iijima and Sibaoka, 1983) the loss per unit area is:

(374 pmole/trap) / (0.38 cm^2/trap) = 984 pmole/cm^2 K^+ per trap

An approximately equal amount of anion would also be lost from the trap. It can be estimated that the total solute lost during trap closure is at least twice as much as the potassium loss:

2 X 374 pmole = 748 pmole solute/trap
2 X 984 pmole/cm^2 = 1968 pmole solute/cm^2

If all loss is through the 0.06 cm^2 motor cells (Fig. 1, Iijima and Sibaoka,1983) we can compute:

(748 pmole solute/trap) / 0.06 cm^2 = 6,233 pmole of K^+/cm^2 or 12,466 pmole of solute/cm^2

Let us now examine each of Iijima's main points using both his assumption that all K^+ loss is through the cells of the motor zone and a second model where the K^+ loss is assumed to be evenly spread over the active area of the trap.

1. Potassium release during the action potential and trap closure. A single action potential will result in the closure of *Aldrovanda*. All action potentials involve the passage of specific ions across the membrane — that is the mechanism by which action potentials occur. Typically both plant and animal action potentials lose potassium ion during the recovery simply as a part of the signal producing process. The loss of potassium would be expected regardless of the mechanism of trap closure. There are two questions that arise:

- Is the K^+ loss during the action potential just that expected from the action potential or is there another mechanism of K^+ loss?
- Regardless of the mechanism, is enough K^+ (and accompanying anion) lost to cause a turgor change in the motor cells sufficient to cause trap closure?

Iijima and Sibaoka (1983) state "It is unlikely that as much as 0.25% of the total potassium can move from the cells during an action potential." Presumably they mean by this that the mechanism of the action potential triggers a flow of potassium ions from the trap that is greater than that to be expected from the action potential itself. But is it "unlikely that as much as 0.25% of the total potassium can move from the cells during an action potential"? Assuming all potassium movement will occur through the motor cells, Iijima and Sibaoka (1983) compute a potassium flow (outward movement) of 6,200 pmole/cm^2 across the membranes of the cells of the motor zone of *Aldrovanda* during an action potential. If they had assumed the flux was across the entire active part of the trap, as an action potential would be expected to do, their figure would have been 984 pmole/cm^2 (Fig 1). Since Oda (1976) measured an outward flow of 1,777 pmole/cm^2 across the membrane of *Chara* (a giant algal cell) during an action potential, it is unnecessary to postulate any mechanism beyond the loss of potassium during the action potential to account for the estimated flux since assuming an efflux of potassium where one is expected from the action potential gives a value smaller than that measured in another plant. There is no reason to suppose that it is unlikely that the

efflux due to the action potential can account for the potassium that leaves the trap during closure.

The question of whether the turgor change in the motor cells resulting from K^+ loss is sufficient to cause movement is the one of major interest. Iijima and Sibaoka (1985) measure 117 meq of chloride ion in trap lobes. Allowing for 117 meq of positive ions to balance the charge there are at least 234 mosmole of solute/liter in the cells. There are likely to be at least 250 mosmole/liter. The osmotic pressure in such a cell would be:

π_{cells} = cRT = 250 osmole/liter X 24.8 liter-bar/mole = 6.2 bar

Another estimate of the osmotic pressure can be computed from the amount of mannitol APW that Iijima and Sibaoka (1983) report is isotonic to the trap (200 mosmolar mannitol +14.5 mosmolar APW) would show:

π_{cells} = cRT = .2145 osmole/liter X 24.8 liter-bar/mole = 5.3 bar

A bar is a metric unit of about one atmosphere, so the cells would have about 6 atmospheres of osmotic pressure.

Iijima and Sibaoka (1983) assume that all the loss of K^+ would be through the motor cells in their calculations (Fig. 1). This results in a high estimate for the amount of turgor lost by the motor cells since these cells compose only 3.2% of the trap volume. A second estimate assuming equal loss from all cells results in a far lower turgor loss by the motor cells. Without knowing from which cells the K^+ is actually lost, it is impossible to say what the actual turgor change in the motor cells is but the true value is likely to lie within the range bounded by these two values.

Assuming equal distribution of loss of trap potassium and an accompanying anion would result in a drop in concentration of:

748 pmole solute/trap
748 pmole/μliter = 0.748 mM

This should result in a change in osmotic pressure of:

π = cRT = 7.48 X 10^{-4} osmole/liter X 24.8 liter-bar/mole = 0.0186 bar

The change in concentration during closure due to the loss of potassium and a balancing charge should result in the loss of about 0.02 atmosphere of osmotic potential in a cell with about 6.0 atmospheres of osmotic potential. If we make the assumption that all the potassium is lost from the cells of the motor zone we can estimate that the internal osmotic potential change will be about 31 times larger by using Iijima and Sibaoka's (1983) estimate that the motor cells constitute 3.2% of the volume of the trap. This would give a value of 0.62 bar, a change of about 10% in the osmotic potential in the motor cells and clearly more than enough to cause a substantial turgor change in the motor cells. In both instances the osmotic pressure ($= \pi_{in} - \pi_{out}$) is influenced by the buildup of the potassium ions lost from the cell outside the cell so the values must be considered the maximum pressure change that could be expected under the circumstances measured. Unfortunately there is no evidence as to which cells are losing the K^+. The loss could be nearly equal from all cells, in which case the K^+ loss from an action potential can easily explain the results, or the loss could be primarily (50 or 75%) from the motor cells, in which case a substantial turgor change would be expected. Even if the loss of K^+ is evenly distributed and due entirely to the mechanism of the action potential, a turgor event cannot be ruled out. In *Chara* internodal cells, bathed in APW, the chloride and potassium ions lost during an action potential cause an 0.38 to 0.55 mm decrease in the length of 70 - 110 mm long cells (Oda and Linstead, 1975). The much larger surface to volume ratio of *Aldrovanda* trap cells would enhance this effect. In the delicately balanced system of the *Aldrovanda* trap these osmotic changes may be enough to cause adequate water movements to trigger closure by Ashida's (1934)

mechanism. It is possible, but not by any means proven, that the initial phases of *Aldrovanda* trap movements result from a turgor mechanism

2. Potassium uptake during the reopening of the trap. The increased uptake of potassium during the reopening of the trap is consistent with what would be expected from a turgor mechanism since restoration of the solute with its accompanying osmotic pressure would cause water to move into the cells and restore turgor. An increased uptake of potassium during the reopening is also consistent with what would be expected from an acid growth mechanism since the expanded cells on the outer lobe would lose turgor and ordinary homeostatic mechanisms which control turgor would be expected to restore turgor to these cells by taking up additional ions from the environment. Here we have data that is expected for both mechanisms and does not distinguish between either of these hypotheses. The increased rate of uptake of potassium after closure is, however, not consistent with a turgor gain mechanism such as that proposed by Brown (1916) for *Dionaea*.

3. Traps placed in a nearly isotonic medium will not close when stimulated to produce an action potential. According to Iijima and Sibaoka (1983) a 215 mM solution is "nearly isotonic" to traps. Triggering an action potential in this solution will not cause trap closure. This observation is consistent with all three hypotheses. If water can not move into or out of trap cells, movement by both turgor gain and loss are impossible. Wall loosening expected in the acid growth mechanism could occur but the cell expansion that causes the movement in this mechanism could not. The observed result is predicted by all three hypotheses and thus the experiment fails to distinguish between them.

When transferred back to a dilute ionic solution (APW) immediately after triggering an action potential in the 200 mM mannitol-APW solution which prevents closure, the traps still remained open. This is a result that is hard to explain with any of the hypotheses. A turgor loss mechanism by rapid diffusion of potassium and an anion (presumably chloride) through specific channels in the membrane should not be prevented by mannitol.

The ion flux should still occur but without the water movements since the effect of the osmotic pressure is rendered a negligible part of the water potential difference which would drive the water movement. Restoration to the dilute solution (APW) should allow the water movement and closure. A parallel argument would hold for a turgor gain mechanism. The growth mechanism should also close after moving the trap to a dilute solution since the water can move into the cells with loosened walls and result in the expansion of the trap. Oddly, we are faced here with a situation where none of the hypotheses seem to explain the results. Ashida (1934) had reported similar results with sugar solutions but also noted that both plant's traps remain shut if placed in sugar solutions after closure. This data is consistent with the acid growth model but not with either turgor mechanism.

Iijima and Sibaoka (1983) suggest that their results from experiments with mannitol-APW solutions are consistent with the hypothesis that there is a bulk flow from the vacuole of the motor cells to the outside of the cell, and that this causes the movement rather than a diffusional flux of ions. They state that this flow must be dependent on pressure being maintained inside the cells. This may be so, but further work needs to be done to test this new fourth hypothesis. An unknown pressure-dependent component could be hypothesized for any of the other three to explain the results, ie., either the potassium channels or the hydrogen ion pump is pressure-sensitive in its response.

Iijima and Sibaoka (1983) have added a hypothesis to the list of possible mechanisms of *Aldrovanda* trap closure and they have provided useful data that indicate a possible role for K^+-driven turgor loss but they have not convincingly demonstrated by what mechanism the trap moves.

In work communicated to me in a letter but never published, Toshio Iijima found that the outer epidermis of the motor region of *Aldrovanda* traps expands 15% and the inner epidermis shrinks by 17%. This would support the action of a combined turgor response and growth response. In his letter he also reported responses to neutral buffers similar to those seen by Alan Bennett and me (Williams and Bennett, 1982) but reported complications with the experiments with acetate buffers due to triggering of action potentials and lowering of cell ATP by acetate. He correctly pointed out in his letter that the closure of *Aldrovanda* trap is 10 to 20 times faster than that of the *Dionaea* trap and that the movement begins when the action potential is still in its fast rising phase. Ashida (1934) measured movement as soon as 80 msec after a generalized electrical stimulus, with substantial movement by 90 msec after the stimulus, while Iijama and Sibaoka (1981) show the action potential's rising phase lasts 200 msec. During this phase a calcium influx (Iijama and Sibaoka, 1985) and possibly a chloride efflux and the beginnings of a potassium efflux are occurring if the mechanism is similar to that in *Chara* (Oda, 1976). It is in this very early period that a turgor loss from the outer motor cells could release a movement that is largely like triggering a spring trap. Ashida (1934) noted that the outer epidermis is undulated as if it were a coiled spring. After closure, the midrib reverses its position and the undulations disappear. The *Aldrovanda* trap may be "cocked" and it may "spring" when a relatively small change occurs —presumably in the inner epidermis. In *Dionaea* the lobes reverse from a convex internal curvature to a concave internal curvature during closure. This produces a mechanical amplification of the movements but the degree may not be nearly as great as in *Aldrovanda.*

Ashida (1934) divided *Aldrovanda* trap closure into "quick phase" closing, "slow phase" closing and trap "narrowing". He believed the quick phase closing was due to turgor loss and that narrowing was due to an increase in the plasticity of the outer walls of the trap. He had qualitative evidence for each mechanism involving observation of the relative degree of undulation of the trap epidermis. He did not clearly specify the mechanism of slow phase closing, but most of it occurred between 0.2 sec and 30 sec, declining to nearly nothing within 60 sec. This is about the rate at which the *Dionaea* trap closes. Perhaps the "slow phase" movement of *Aldrovanda* closure is caused by wall loosening by an acid growth mechanism similar to that in *Dionaea.* Both plants would then have the same physiological events going on in their cells but in *Dionaea* the osmotic effects of ion loss during the action potential may be negligible in their effect on the movement.

Toshio Iijima, Takao Sibaoka and Joji Ashida have advanced our understanding of *Aldrovanda* trap to a point where its electrophysiology and trapping movements are among the best understood of any of the carnivorous plants, partly because this plant's aquatic nature lends it to this type of study and largely because of a considerable amount of incredibly careful and exacting work. This discussion only deals with one important phase of the work of these men.

Thanks are due to Dr. John D. DeGreef of Liège, Belgium and Dr. Donald Dahlberg of the Chemistry Department, Lebanon Valley College for reviewing the manuscript.

Literature Cited

Ashida, J. (1934), Mem. Coll. Sci. Univ. Kyoto, Ser. B 11: 55-113. Brown, W. (1916) Am. J. Bot. 3: 68-90.
Iijima, T. and T. Sibaoka (1981) Plant and Cell Physiol. 22: 1595-1601.
Iijima, T. and T. Sibaoka (1982) Plant and Cell Physiol. 23: 679-688.
Iijima, T. and T. Sibaoka (1983) Plant and Cell Physiol. 24: 51-60.
Iijima, T. and T. Sibaoka (1985) Plant and Cell Physiol. 26: 1-13.
Oda, K. (1976), Plant and Cell Physiol. 17: 1085-1088.

Oda, K. and J. Linstead, (1975), J. Exp. Bot. 26: 228-239.
Williams, S. (1991) Carnivorous Plant Newsletter, In Press
Williams, S. and A. Bennett (1982) Science 218: 1120-1122.

[1] You can test the turgidity yourself with a probe, such as a ballpoint pen. Push the probe gently into the epidermis of each side of an open trap. If your plant is well watered it will be turgid (hard). Close the trap by stimulating the hairs twice. Probe the trap soon after closure. It will be flaccid (soft) and your probe will sink in a bit. Cut the trap down the center and probe the inside. It will be turgid. This is what the acid growth mechanism predicts but it is the opposite of what a turgor mechanism would predict.

[2] Mannitol is a sugar alcohol often used in experiments on osmotic pressure. APW stands for "artificial pond water" and is often used in electrophysiological experiments on freshwater and terrestrial plants.

A Letter From Sierra Leone[1]

Dear CPS:

I hope all is well, I hear there's been quite a hard winter, not too many casualties I hope.

After a long, *Triphyophyllum peltatum* filled silence, I write again (I would like to think more coherently than my last tropically stunned communication).

I have been in West Africa some months now, although my time in 'Tome' land (the plant's name in Mende - one of the local tribes - and in fact the only one of S. Leone's ten or so that has a word for it) is split into two by a Christmas gadabout in Mali. Before leaving the country for Christmas then, I went around bush paths, small villages (they are spiders with leg paths) going nowhere in particular enquiring about the plant. This was some adventure in itself - in villages in the south-western part of the country, it is not widespread and so only the elders who had made studies of plants, usually medicine men or witch doctors, knew about it, and my investigations were often met with some difficulties - sometimes it would be growing in sacred society bush, which a non-member cannot enter ("coco jinoku, mahanhoo mahmoo gonge" is a Mende proverb which means something like "We who are in the Society and know, are not going to tell you, so nur nur-ne nur nur", dispensed freely to those who ask too many questions). Another problem was a fierce old man with a bald head save for a few white popples of hair, who demanded in payment for the knowledge of a medicine man, that I should marry his daughter.

The first time I did see the plant I was unimpressed - a blackish liana twizzling up into the sky via an ant-covered giant tree. Somewhere up there I fancied, just maybe, I could see the characteristic tendrilled leaves; climbing was out though.

As I moved further eastwards, the plant became more common, as far as I could tell as the surrounding bush changed from heavily cultivated farm bush type to predominantly old secondary forest with patches of farms and patches of primary forest. It was about here that people would proudly announce that they had seen "Tome" on their farms - and show it to me before cutting it down with surrounding trees ("brushing") to make room for next year's rice crop.

All I saw, despite huge searches, was the mature lianas, or the seedling in the first growth stage. I began to remember that many botanical hoaxes have concerned carnivorous plants, but dutifully planted a nursery bed by a stream to return to at a later date! (Actually, I don't know where I remembered that from, I probably made it up. It's the sun you know)